

SUPPLEMENT

Is There a Critical Period for the Developmental Neurotoxicity of Low-Level Tobacco Smoke Exposure?

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TABLE S1: Body and Brain Region Weights (mean \pm SE)

	Age	Male				Female			
		Control	TSE Premating	TSE Early Gestation	TSE Late Gestation	Control	TSE Premating	TSE Early Gestation	TSE Late Gestation
Body Weight (g)	30	116 \pm 1	115 \pm 2	110 \pm 2	112 \pm 2	102 \pm 6	103 \pm 3	96 \pm 1	106 \pm 1
	60	390 \pm 5	384 \pm 2	392 \pm 7	382 \pm 3	235 \pm 2	229 \pm 3	228 \pm 2	239 \pm 4
	100	557 \pm 10	550 \pm 7	559 \pm 10	540 \pm 7	295 \pm 9	293 \pm 8	279 \pm 8	297 \pm 7
	150	667 \pm 13	639 \pm 19	645 \pm 19	660 \pm 10	334 \pm 10	299 \pm 5	313 \pm 5	321 \pm 5
Region Weight (mg)									
frontal/parietal cortex	30	237 \pm 6	228 \pm 4	222 \pm 10	236 \pm 12	225 \pm 3	238 \pm 4	220 \pm 9	224 \pm 12
	60	256 \pm 9	262 \pm 5	264 \pm 5	260 \pm 14	235 \pm 6	244 \pm 8	244 \pm 7	242 \pm 9
	100	269 \pm 7	264 \pm 8	262 \pm 10	256 \pm 9	260 \pm 14	252 \pm 6	238 \pm 10	234 \pm 4
	150	274 \pm 11	251 \pm 6	253 \pm 10	275 \pm 12	231 \pm 9	240 \pm 5	244 \pm 8	244 \pm 7
temporal/occipital cortex	30	176 \pm 4	188 \pm 15	176 \pm 5	174 \pm 8	166 \pm 9	171 \pm 9	167 \pm 5	169 \pm 5
	60	202 \pm 5	201 \pm 7	196 \pm 10	191 \pm 8	186 \pm 5	187 \pm 7	182 \pm 6	183 \pm 7
	100	222 \pm 10	230 \pm 15	211 \pm 8	213 \pm 13	219 \pm 20	201 \pm 19	197 \pm 3	220 \pm 9
	150	208 \pm 4	203 \pm 7	212 \pm 12	204 \pm 8	205 \pm 9	189 \pm 7	189 \pm 8	197 \pm 4
hippocampus	30	129 \pm 6	133 \pm 4	125 \pm 3	126 \pm 3	124 \pm 7	125 \pm 6	121 \pm 2	130 \pm 6
	60	153 \pm 7	147 \pm 6	156 \pm 5	148 \pm 6	142 \pm 4	146 \pm 3	149 \pm 3	140 \pm 7
	100	163 \pm 3	169 \pm 7	155 \pm 3	160 \pm 4	156 \pm 9	147 \pm 6	159 \pm 8	147 \pm 5
	150	158 \pm 5	164 \pm 7	161 \pm 6	170 \pm 7	150 \pm 6	151 \pm 4	153 \pm 12	149 \pm 6
striatum	30	90 \pm 6	94 \pm 6	90 \pm 6	96 \pm 6	93 \pm 5	95 \pm 6	79 \pm 5	104 \pm 9
	60	113 \pm 8	115 \pm 2	120 \pm 8	119 \pm 9	110 \pm 11	103 \pm 5	110 \pm 5	100 \pm 5
	100	112 \pm 5	131 \pm 11	130 \pm 9	124 \pm 5	106 \pm 5	112 \pm 4	120 \pm 9	104 \pm 9
	150	120 \pm 8	119 \pm 5	114 \pm 5	124 \pm 4	113 \pm 8	104 \pm 4	104 \pm 7	104 \pm 6
midbrain	30	272 \pm 13	280 \pm 8	270 \pm 4	264 \pm 3	260 \pm 4	256 \pm 12	257 \pm 2	264 \pm 7
	60	333 \pm 10	346 \pm 6	321 \pm 6	346 \pm 11	307 \pm 5	307 \pm 5	297 \pm 18	308 \pm 8
	100	355 \pm 10	367 \pm 7	351 \pm 7	348 \pm 15	332 \pm 10	330 \pm 10	334 \pm 7	331 \pm 4
	150	376 \pm 9	380 \pm 6	374 \pm 7	362 \pm 7	341 \pm 12	346 \pm 7	338 \pm 9	335 \pm 14
brainstem	30	156 \pm 1	152 \pm 4	148 \pm 4	153 \pm 3	152 \pm 5	152 \pm 5	150 \pm 6	147 \pm 11
	60	223 \pm 4	216 \pm 9	219 \pm 8	218 \pm 7	213 \pm 4	204 \pm 10	215 \pm 17	210 \pm 5
	100	265 \pm 11	249 \pm 10	255 \pm 10	239 \pm 7	233 \pm 3	237 \pm 5	233 \pm 6	238 \pm 5
	150	269 \pm 4	256 \pm 8	258 \pm 9	266 \pm 7	240 \pm 10	233 \pm 5	237 \pm 6	247 \pm 5

Data represent mean \pm SE obtained from six animals in each treatment group for each age and sex. For body weight, three-factor ANOVA (treatment, age, sex) indicates a main effect of treatment ($p < 0.0009$) as well as interactions of treatment \times sex ($p < 0.02$) and treatment \times age ($p < 0.04$). After subdivision by sex, males showed no significant weight differences, whereas females did (main treatment effect, $p < 0.0002$); for females, the TSE Premating and TSE Early Gestation groups were significantly different from control ($p < 0.03$ and $p < 0.0006$, respectively), representing average deficits of 3-5%. After subdivision by age, we found a significant treatment effect restricted to PN30 ($p < 0.002$), with the TSE Early Gestation group as the only one showing a significant difference from control (5% deficit, $p < 0.002$). Further subdivisions were not assessed because of the absence of a treatment \times sex \times age interaction; likewise, there were no significant treatment \times sex interactions at any of the individual age points, nor any treatment \times age interaction for either sex.

For brain region weight, four-factor ANOVA (treatment, age, sex, region) indicates no significant treatment effects and no interactions of treatment with the other factors. Note that weights for frontal/parietal cortex and temporal/occipital cortex are for the right hemisphere only, the portion used in this study.

TABLE S2: ChAT Activity (mean \pm SE)

Region	Postnatal Age	Male (pmol/min per mg protein)				Female (pmol/min per mg protein)			
		Control	TSE Premating	TSE Early Gestation	TSE Late Gestation	Control	TSE Premating	TSE Early Gestation	TSE Late Gestation
frontal/parietal cortex	30	0.69 \pm 0.04	0.74 \pm 0.03	0.78 \pm 0.03	0.77 \pm 0.03	0.76 \pm 0.02	0.80 \pm 0.03	0.77 \pm 0.05	0.72 \pm 0.02
	60	0.77 \pm 0.06	0.81 \pm 0.02	0.82 \pm 0.03	0.73 \pm 0.03	0.80 \pm 0.06	0.78 \pm 0.02	0.76 \pm 0.03	0.81 \pm 0.02
	100	0.73 \pm 0.03	0.76 \pm 0.04	0.73 \pm 0.02	0.72 \pm 0.06	0.73 \pm 0.03	0.75 \pm 0.03	0.74 \pm 0.02	0.69 \pm 0.05
	150	0.78 \pm 0.02	0.85 \pm 0.03	0.78 \pm 0.01	0.79 \pm 0.02	0.86 \pm 0.03	0.87 \pm 0.03	0.82 \pm 0.03	0.84 \pm 0.04
temporal/occipital cortex	30	0.78 \pm 0.03	0.78 \pm 0.05	0.88 \pm 0.05	0.89 \pm 0.05	0.73 \pm 0.02	0.87 \pm 0.03	0.85 \pm 0.04	0.77 \pm 0.05
	60	0.47 \pm 0.02	0.49 \pm 0.02	0.47 \pm 0.02	0.46 \pm 0.02	0.50 \pm 0.01	0.47 \pm 0.02	0.48 \pm 0.04	0.52 \pm 0.02
	100	0.53 \pm 0.01	0.55 \pm 0.03	0.57 \pm 0.02	0.59 \pm 0.03	0.52 \pm 0.03	0.57 \pm 0.02	0.59 \pm 0.02	0.58 \pm 0.02
	150	0.67 \pm 0.03	0.76 \pm 0.05	0.76 \pm 0.02	0.69 \pm 0.01	0.73 \pm 0.02	0.77 \pm 0.02	0.74 \pm 0.02	0.76 \pm 0.04
hippocampus	30	0.89 \pm 0.04	0.83 \pm 0.04	0.94 \pm 0.07	0.86 \pm 0.05	0.91 \pm 0.02	0.94 \pm 0.06	0.91 \pm 0.07	0.83 \pm 0.03
	60	0.91 \pm 0.04	0.90 \pm 0.04	0.90 \pm 0.05	0.88 \pm 0.03	0.94 \pm 0.04	1.02 \pm 0.05	0.87 \pm 0.03	0.92 \pm 0.06
	100	1.06 \pm 0.06	1.16 \pm 0.03	1.09 \pm 0.07	1.10 \pm 0.04	1.08 \pm 0.06	1.20 \pm 0.04	1.13 \pm 0.04	0.99 \pm 0.03
	150	1.02 \pm 0.02	1.07 \pm 0.02	1.06 \pm 0.04	1.00 \pm 0.03	1.11 \pm 0.03	1.11 \pm 0.02	1.10 \pm 0.03	1.06 \pm 0.04
striatum	30	2.27 \pm 0.12	2.27 \pm 0.05	2.61 \pm 0.09	2.43 \pm 0.11	2.34 \pm 0.09	2.23 \pm 0.11	2.75 \pm 0.19	2.32 \pm 0.16
	60	2.61 \pm 0.13	2.98 \pm 0.07	2.70 \pm 0.11	2.82 \pm 0.05	3.09 \pm 0.16	2.79 \pm 0.12	3.02 \pm 0.09	2.43 \pm 0.11
	100	2.02 \pm 0.12	2.25 \pm 0.08	2.57 \pm 0.13	2.27 \pm 0.10	2.47 \pm 0.07	2.43 \pm 0.16	2.36 \pm 0.09	2.35 \pm 0.05
	150	2.44 \pm 0.20	2.57 \pm 0.15	2.67 \pm 0.10	2.36 \pm 0.11	2.43 \pm 0.09	2.63 \pm 0.12	2.52 \pm 0.11	2.28 \pm 0.16
midbrain	30	0.63 \pm 0.04	0.66 \pm 0.02	0.74 \pm 0.04	0.68 \pm 0.03	0.70 \pm 0.03	0.68 \pm 0.03	0.73 \pm 0.04	0.63 \pm 0.02
	60	0.64 \pm 0.03	0.65 \pm 0.03	0.65 \pm 0.02	0.65 \pm 0.01	0.65 \pm 0.01	0.67 \pm 0.02	0.70 \pm 0.02	0.65 \pm 0.02
	100	0.61 \pm 0.03	0.61 \pm 0.02	0.63 \pm 0.03	0.64 \pm 0.03	0.61 \pm 0.01	0.70 \pm 0.02	0.67 \pm 0.02	0.61 \pm 0.03
	150	0.62 \pm 0.02	0.68 \pm 0.02	0.72 \pm 0.03	0.70 \pm 0.02	0.72 \pm 0.02	0.72 \pm 0.02	0.71 \pm 0.02	0.64 \pm 0.03
brainstem	30	1.14 \pm 0.02	1.16 \pm 0.03	1.21 \pm 0.03	1.15 \pm 0.04	1.16 \pm 0.03	1.16 \pm 0.03	1.16 \pm 0.03	1.16 \pm 0.05
	60	1.06 \pm 0.03	1.10 \pm 0.03	1.13 \pm 0.04	1.07 \pm 0.04	1.12 \pm 0.02	1.13 \pm 0.03	1.15 \pm 0.05	1.13 \pm 0.03
	100	0.95 \pm 0.06	0.98 \pm 0.02	1.02 \pm 0.03	1.03 \pm 0.04	0.99 \pm 0.03	1.06 \pm 0.03	1.01 \pm 0.02	1.00 \pm 0.02
	150	1.11 \pm 0.05	1.11 \pm 0.03	1.12 \pm 0.02	1.05 \pm 0.03	1.15 \pm 0.04	1.20 \pm 0.03	1.09 \pm 0.04	1.08 \pm 0.03

Data represent mean \pm SE obtained from six animals in each treatment group for each age and sex. Results of multivariate ANOVA are provided in the main text. Note that the assays for each region and age were run in separate experiments, so absolute values cannot be compared strictly across ages or between regions. Accordingly, statistical comparisons in the main text were conducted on log-transformed data, which evaluates the treatment differences as a proportion to control values, rather than as an arithmetic difference. Representing the data as proportional differences (percent control) enables a full comparison of treatment effects and treatment interactions with all the other variables, even though absolute values for the controls cannot be compared across regions and ages.

TABLE S3: HC3 Binding (mean \pm SE)

Region	Postnatal Age	Male (fmol/mg protein)				Female (fmol/mg protein)			
		Control	TSE Premating	TSE Early Gestation	TSE Late Gestation	Control	TSE Premating	TSE Early Gestation	TSE Late Gestation
frontal/parietal cortex	30	10.6 \pm 0.8	8.8 \pm 0.5	9.3 \pm 0.5	9.2 \pm 0.3	10.8 \pm 1.1	8.7 \pm 0.4	9.4 \pm 0.4	8.3 \pm 0.5
	60	13.1 \pm 0.6	12.7 \pm 0.7	11.7 \pm 0.7	10.8 \pm 0.8	13.4 \pm 0.4	11.5 \pm 0.7	11.7 \pm 0.9	12.6 \pm 0.9
	100	7.7 \pm 0.4	7.5 \pm 0.6	7.1 \pm 0.7	6.4 \pm 0.5	7.5 \pm 0.6	8.3 \pm 1.1	7.3 \pm 0.4	6.7 \pm 0.6
	150	10.4 \pm 0.8	11.3 \pm 0.5	9.1 \pm 0.7	9.7 \pm 0.5	10.6 \pm 0.4	11.2 \pm 0.6	9.3 \pm 0.4	9.6 \pm 0.6
temporal/occipital cortex	30	7.3 \pm 0.7	7.2 \pm 0.3	6.5 \pm 0.3	6.8 \pm 0.5	6.4 \pm 0.7	6.6 \pm 0.4	5.8 \pm 0.6	6.3 \pm 0.6
	60	6.7 \pm 0.2	6.0 \pm 0.5	5.5 \pm 0.3	6.2 \pm 0.4	7.4 \pm 0.4	6.8 \pm 0.4	6.0 \pm 0.4	5.5 \pm 0.7
	100	7.1 \pm 0.3	6.7 \pm 0.3	6.4 \pm 0.4	6.8 \pm 0.4	6.8 \pm 0.3	6.7 \pm 0.4	7.7 \pm 0.6	6.2 \pm 0.5
	150	7.0 \pm 0.4	7.6 \pm 0.3	6.5 \pm 0.3	6.3 \pm 0.8	7.2 \pm 0.4	7.8 \pm 0.5	6.0 \pm 0.3	7.0 \pm 0.2
hippocampus	30	10.1 \pm 0.8	9.4 \pm 0.6	8.3 \pm 0.5	7.4 \pm 1.1	10.4 \pm 0.9	7.9 \pm 0.4	9.0 \pm 0.5	6.6 \pm 0.6
	60	8.9 \pm 0.5	8.7 \pm 0.5	9.4 \pm 0.6	8.8 \pm 0.4	9.4 \pm 0.8	8.8 \pm 1.0	8.8 \pm 0.5	7.4 \pm 0.6
	100	12.7 \pm 0.6	12.9 \pm 0.7	11.3 \pm 0.7	11.4 \pm 0.8	12.1 \pm 0.9	11.5 \pm 0.9	12.4 \pm 1.0	11.3 \pm 0.7
	150	12.2 \pm 0.4	11.8 \pm 0.8	10.0 \pm 0.3	9.8 \pm 0.4	11.9 \pm 0.4	11.8 \pm 1.0	11.5 \pm 0.9	11.9 \pm 0.6
striatum	30	46 \pm 3	54 \pm 5	46 \pm 2	48 \pm 3	49 \pm 2	50 \pm 2	55 \pm 5	43 \pm 2
	60	60 \pm 8	52 \pm 4	40 \pm 3	43 \pm 2	49 \pm 5	42 \pm 4	48 \pm 4	40 \pm 4
	100	48 \pm 4	56 \pm 4	47 \pm 4	41 \pm 4	49 \pm 4	50 \pm 2	49 \pm 4	43 \pm 3
	150	37 \pm 1	41 \pm 2	36 \pm 3	36 \pm 3	44 \pm 3	38 \pm 3	38 \pm 2	36 \pm 3
midbrain	30	6.4 \pm 0.4	6.7 \pm 0.5	6.7 \pm 0.3	6.9 \pm 0.5	7.4 \pm 0.3	6.3 \pm 0.4	6.4 \pm 0.3	5.9 \pm 0.3
	60	9.2 \pm 0.4	9.3 \pm 0.4	8.3 \pm 0.5	7.5 \pm 0.4	8.8 \pm 0.4	8.7 \pm 0.4	7.4 \pm 0.6	7.6 \pm 0.6
	100	6.9 \pm 0.5	6.4 \pm 0.5	6.4 \pm 0.3	6.5 \pm 0.5	6.3 \pm 0.2	6.8 \pm 0.4	6.7 \pm 0.4	6.6 \pm 0.3
	150	6.9 \pm 0.3	7.5 \pm 0.2	6.7 \pm 0.2	7.0 \pm 0.3	7.5 \pm 0.4	7.3 \pm 0.2	7.2 \pm 0.5	6.6 \pm 0.2
brainstem	30	5.9 \pm 0.9	5.9 \pm 0.8	5.7 \pm 0.3	5.4 \pm 0.3	7.2 \pm 0.8	6.0 \pm 0.4	5.2 \pm 0.3	5.5 \pm 0.9
	60	3.7 \pm 0.2	3.6 \pm 0.2	3.3 \pm 0.1	3.0 \pm 0.2	3.6 \pm 0.1	3.8 \pm 0.4	3.0 \pm 0.2	3.0 \pm 0.4
	100	3.7 \pm 0.4	3.7 \pm 0.5	4.0 \pm 0.4	3.9 \pm 0.3	3.6 \pm 0.4	3.3 \pm 0.4	3.0 \pm 0.3	3.0 \pm 0.2
	150	3.4 \pm 0.2	3.5 \pm 0.2	3.5 \pm 0.2	3.9 \pm 0.1	3.9 \pm 0.1	3.4 \pm 0.1	3.8 \pm 0.2	3.6 \pm 0.2

Data represent mean \pm SE obtained from six animals in each treatment group for each age and sex. Results of multivariate ANOVA are provided in the main text. Note that the assays for each region and age were run in separate experiments, so absolute values cannot be compared strictly across ages or between regions. Accordingly, statistical comparisons in the main text were conducted on log-transformed data, which evaluates the treatment differences as a proportion to control values, rather than as an arithmetic difference. Representing the data as proportional differences (percent control) enables a full comparison of treatment effects and treatment interactions with all the other variables, even though absolute values for the controls cannot be compared across regions and ages.

TABLE S4: HC3/ChAT ratio (mean \pm SE)

Region	Postnatal Age	Male				Female			
		Control	TSE Premating	TSE Early Gestation	TSE Late Gestation	Control	TSE Premating	TSE Early Gestation	TSE Late Gestation
frontal/parietal cortex	30	15.5 \pm 1.4	12.3 \pm 0.6	11.8 \pm 0.5	12.3 \pm 0.6	14.2 \pm 1.5	11.0 \pm 0.7	12.3 \pm 0.7	11.5 \pm 0.6
	60	17.4 \pm 1.5	15.7 \pm 0.8	14.3 \pm 0.6	14.9 \pm 0.9	17.0 \pm 0.8	14.8 \pm 0.8	15.4 \pm 1.1	15.5 \pm 0.8
	100	10.5 \pm 0.4	10.0 \pm 0.8	9.7 \pm 0.9	9.0 \pm 0.7	10.3 \pm 0.6	10.7 \pm 1.1	9.8 \pm 0.6	9.6 \pm 0.5
	150	13.3 \pm 0.9	13.4 \pm 0.6	11.7 \pm 0.9	12.3 \pm 0.5	12.5 \pm 0.6	12.9 \pm 0.4	11.4 \pm 0.8	11.4 \pm 0.6
temporal/occipital cortex	30	9.4 \pm 0.9	9.1 \pm 0.3	7.5 \pm 0.5	7.7 \pm 0.7	8.7 \pm 0.8	7.5 \pm 0.4	6.8 \pm 0.5	8.2 \pm 0.5
	60	14.9 \pm 0.5	11.9 \pm 0.7	11.7 \pm 0.6	13.5 \pm 1.0	15.0 \pm 0.8	14.6 \pm 0.8	12.9 \pm 1.4	10.8 \pm 1.2
	100	13.5 \pm 0.6	12.2 \pm 0.6	11.3 \pm 0.6	11.7 \pm 0.6	13.6 \pm 1.5	11.7 \pm 0.9	13.2 \pm 1.1	10.8 \pm 0.5
	150	10.5 \pm 0.6	10.3 \pm 0.7	8.5 \pm 0.3	8.3 \pm 0.9	9.8 \pm 0.6	10.2 \pm 0.6	8.3 \pm 0.6	9.5 \pm 0.8
hippocampus	30	11.5 \pm 1.2	11.5 \pm 1.1	9.0 \pm 0.7	9.0 \pm 1.2	11.4 \pm 1.3	8.4 \pm 0.2	10.0 \pm 0.6	7.8 \pm 0.8
	60	9.9 \pm 0.7	9.8 \pm 0.8	10.1 \pm 1.0	10.2 \pm 0.9	10.0 \pm 0.7	8.7 \pm 1.1	10.1 \pm 0.6	8.3 \pm 0.9
	100	11.5 \pm 0.2	11.2 \pm 0.6	10.7 \pm 1.0	10.3 \pm 0.7	11.3 \pm 0.9	9.7 \pm 0.8	11.0 \pm 0.9	11.1 \pm 0.9
	150	12.0 \pm 0.4	10.7 \pm 0.7	9.3 \pm 0.3	10.2 \pm 0.6	10.6 \pm 0.4	10.6 \pm 1.0	10.4 \pm 0.6	11.4 \pm 0.8
striatum	30	20 \pm 1	24 \pm 2	18 \pm 1	20 \pm 2	21 \pm 1	22 \pm 2	20 \pm 1	19 \pm 1
	60	24 \pm 4	17 \pm 1	15 \pm 1	15 \pm 1	16 \pm 2	15 \pm 1	16 \pm 1	16 \pm 1
	100	23 \pm 2	25 \pm 2	19 \pm 2	20 \pm 1	20 \pm 2	21 \pm 2	21 \pm 1	19 \pm 1
	150	16 \pm 1	16 \pm 1	13 \pm 1	15 \pm 1	18 \pm 1	15 \pm 2	15 \pm 1	16 \pm 1
midbrain	30	10.5 \pm 1.3	10.0 \pm 0.6	9.0 \pm 0.4	10.1 \pm 0.7	10.3 \pm 0.8	9.2 \pm 0.7	8.8 \pm 0.5	9.2 \pm 0.5
	60	14.7 \pm 1.0	14.3 \pm 0.5	12.8 \pm 0.6	11.6 \pm 0.6	13.4 \pm 0.8	12.8 \pm 0.5	10.9 \pm 1.0	11.8 \pm 1.0
	100	11.5 \pm 1.0	10.7 \pm 0.8	10.5 \pm 0.5	10.2 \pm 0.8	10.4 \pm 0.3	9.8 \pm 0.8	10.0 \pm 0.6	11.0 \pm 0.6
	150	11.2 \pm 0.5	11.0 \pm 0.4	9.4 \pm 0.5	10.0 \pm 0.4	10.5 \pm 0.6	10.1 \pm 0.3	10.1 \pm 0.8	10.3 \pm 0.5
brainstem	30	5.3 \pm 1.0	5.0 \pm 0.5	4.5 \pm 0.3	4.7 \pm 0.4	6.2 \pm 0.6	5.3 \pm 0.8	4.4 \pm 0.4	4.5 \pm 0.8
	60	3.5 \pm 0.2	3.4 \pm 0.2	2.9 \pm 0.2	2.8 \pm 0.2	3.2 \pm 0.1	3.4 \pm 0.4	2.6 \pm 0.2	2.7 \pm 0.3
	100	3.8 \pm 0.6	3.8 \pm 0.5	3.9 \pm 0.4	3.8 \pm 0.3	3.7 \pm 0.4	3.2 \pm 0.4	2.9 \pm 0.3	3.0 \pm 0.2
	150	3.0 \pm 0.3	3.1 \pm 0.2	3.2 \pm 0.2	3.7 \pm 0.2	3.4 \pm 0.2	2.9 \pm 0.1	3.5 \pm 0.2	3.3 \pm 0.2

Data represent mean \pm SE obtained from six animals in each treatment group for each age and sex. Results of multivariate ANOVA are provided in the main text. Note that the assays for each region and age were run in separate experiments, so absolute values cannot be compared strictly across ages or between regions. Accordingly, statistical comparisons in the main text were conducted on log-transformed data, which evaluates the treatment differences as a proportion to control values, rather than as an arithmetic difference. Representing the data as proportional differences (percent control) enables a full comparison of treatment effects and treatment interactions with all the other variables, even though absolute values for the controls cannot be compared across regions and ages.

TABLE S5: nAChR Binding (mean \pm SE)

Region	Postnatal Age	Male				Female			
		Control	TSE Premating	TSE Early Gestation	TSE Late Gestation	Control	TSE Premating	TSE Early Gestation	TSE Late Gestation
frontal/parietal cortex	30	57 \pm 4	58 \pm 2	53 \pm 2	53 \pm 3	57 \pm 3	53 \pm 2	53 \pm 3	47 \pm 5
	60	66 \pm 4	64 \pm 3	59 \pm 5	57 \pm 5	57 \pm 4	49 \pm 2	59 \pm 4	54 \pm 5
	100	57 \pm 2	58 \pm 3	53 \pm 2	49 \pm 2	51 \pm 2	53 \pm 2	51 \pm 6	47 \pm 3
	150	38 \pm 2	41 \pm 3	35 \pm 5	45 \pm 3	39 \pm 2	39 \pm 3	31 \pm 3	35 \pm 2
temporal/occipital cortex	30	78 \pm 5	76 \pm 2	66 \pm 5	74 \pm 5	81 \pm 5	76 \pm 3	69 \pm 4	64 \pm 3
	60	63 \pm 1	71 \pm 2	60 \pm 2	61 \pm 4	66 \pm 2	57 \pm 1	59 \pm 3	47 \pm 3
	100	49 \pm 2	55 \pm 5	48 \pm 2	47 \pm 2	52 \pm 5	51 \pm 3	51 \pm 5	49 \pm 4
	150	52 \pm 1	45 \pm 1	49 \pm 3	45 \pm 3	51 \pm 1	47 \pm 3	45 \pm 4	44 \pm 3
hippocampus	30	40 \pm 2	44 \pm 4	38 \pm 3	40 \pm 4	41 \pm 2	37 \pm 2	40 \pm 1	28 \pm 3
	60	36 \pm 2	36 \pm 2	35 \pm 4	31 \pm 1	35 \pm 2	35 \pm 2	35 \pm 2	29 \pm 2
	100	37 \pm 2	42 \pm 4	30 \pm 3	35 \pm 2	37 \pm 3	34 \pm 3	35 \pm 4	32 \pm 4
	150	29 \pm 1	33 \pm 2	26 \pm 3	29 \pm 3	36 \pm 2	29 \pm 3	30 \pm 1	28 \pm 2
striatum	30	—	—	—	—	—	—	—	—
	60	78 \pm 2	72 \pm 3	70 \pm 5	74 \pm 6	74 \pm 5	68 \pm 4	75 \pm 5	63 \pm 5
	100	72 \pm 13	84 \pm 6	75 \pm 8	70 \pm 8	77 \pm 12	82 \pm 11	75 \pm 7	63 \pm 5
	150	66 \pm 2	72 \pm 4	66 \pm 4	65 \pm 4	73 \pm 3	61 \pm 5	65 \pm 1	56 \pm 3
midbrain	30	69 \pm 2	61 \pm 2	69 \pm 2	57 \pm 4	68 \pm 2	60 \pm 4	67 \pm 4	63 \pm 1
	60	56 \pm 2	54 \pm 1	50 \pm 2	43 \pm 2	54 \pm 2	50 \pm 3	49 \pm 4	46 \pm 2
	100	54 \pm 4	60 \pm 5	55 \pm 3	53 \pm 3	53 \pm 2	53 \pm 3	52 \pm 4	53 \pm 4
	150	49 \pm 2	49 \pm 1	47 \pm 1	48 \pm 2	55 \pm 2	48 \pm 2	48 \pm 2	49 \pm 3
brainstem	30	34 \pm 1	38 \pm 2	35 \pm 1	33 \pm 2	37 \pm 2	34 \pm 1	32 \pm 2	32 \pm 3
	60	27 \pm 1	26 \pm 1	27 \pm 1	26 \pm 1	25 \pm 1	27 \pm 1	26 \pm 2	25 \pm 1
	100	25 \pm 1	27 \pm 1	24 \pm 1	26 \pm 1	28 \pm 2	30 \pm 1	24 \pm 1	23 \pm 2
	150	25 \pm 2	27 \pm 2	24 \pm 2	26 \pm 1	26 \pm 1	26 \pm 2	24 \pm 1	25 \pm 1

Data represent mean \pm SE obtained from six animals in each treatment group for each age and sex. Results of multivariate ANOVA are provided in the main text. Note that the assays for each region and age were run in separate experiments, so absolute values cannot be compared strictly across ages or between regions. Accordingly, statistical comparisons in the main text were conducted on log-transformed data, which evaluates the treatment differences as a proportion to control values, rather than as an arithmetic difference. Representing the data as proportional differences (percent control) enables a full comparison of treatment effects and treatment interactions with all the other variables, even though absolute values for the controls cannot be compared across regions and ages.

TABLE S6: 5HT Receptor Binding (mean \pm SE)

Subtype and Region	Postnatal Age	Male (fmol/mg protein)				Female (fmol/mg protein)			
5HT _{1A} Receptors		Control	TSE Premating	TSE Early Gestation	TSE Late Gestation	Control	TSE Premating	TSE Early Gestation	TSE Late Gestation
frontal/parietal cortex	30	76 \pm 2	74 \pm 5	65 \pm 6	61 \pm 7	83 \pm 6	76 \pm 3	70 \pm 6	67 \pm 6
	60	107 \pm 4	109 \pm 3	103 \pm 9	90 \pm 6	105 \pm 3	102 \pm 7	105 \pm 9	92 \pm 9
	100	105 \pm 3	110 \pm 3	105 \pm 7	98 \pm 6	100 \pm 10	110 \pm 9	111 \pm 10	101 \pm 8
	150	95 \pm 3	91 \pm 7	83 \pm 8	85 \pm 8	89 \pm 7	93 \pm 8	83 \pm 6	79 \pm 5
temporal/occipital cortex	30	131 \pm 5	129 \pm 10	129 \pm 5	124 \pm 7	138 \pm 14	145 \pm 9	128 \pm 11	135 \pm 9
	60	115 \pm 2	111 \pm 5	112 \pm 7	102 \pm 7	120 \pm 6	111 \pm 6	104 \pm 6	95 \pm 8
	100	92 \pm 5	100 \pm 5	94 \pm 5	93 \pm 8	85 \pm 5	101 \pm 6	99 \pm 5	89 \pm 5
	150	124 \pm 1	110 \pm 7	119 \pm 11	107 \pm 9	116 \pm 6	127 \pm 11	101 \pm 7	115 \pm 8
midbrain	30	67 \pm 2	68 \pm 5	69 \pm 5	64 \pm 7	73 \pm 2	70 \pm 7	71 \pm 4	62 \pm 6
	60	64 \pm 1	61 \pm 4	58 \pm 5	48 \pm 3	59 \pm 4	59 \pm 2	53 \pm 6	51 \pm 4
	100	47 \pm 2	50 \pm 4	43 \pm 4	45 \pm 3	43 \pm 1	43 \pm 3	44 \pm 3	44 \pm 3
	150	51 \pm 5	54 \pm 2	54 \pm 4	53 \pm 5	54 \pm 2	53 \pm 3	50 \pm 1	48 \pm 4
brainstem	30	50 \pm 1	55 \pm 1	49 \pm 1	47 \pm 3	55 \pm 2	53 \pm 1	49 \pm 3	50 \pm 6
	60	33 \pm 2	34 \pm 1	31 \pm 3	27 \pm 1	34 \pm 2	35 \pm 2	29 \pm 2	29 \pm 2
	100	29 \pm 1	31 \pm 2	30 \pm 2	29 \pm 2	30 \pm 2	32 \pm 1	30 \pm 1	26 \pm 3
	150	31 \pm 1	33 \pm 1	29 \pm 2	29 \pm 2	35 \pm 2	31 \pm 1	29 \pm 1	30 \pm 2
5HT ₂ Receptors									
frontal/parietal cortex	30	174 \pm 5	177 \pm 3	178 \pm 4	176 \pm 5	177 \pm 2	177 \pm 4	175 \pm 7	175 \pm 2
	60	194 \pm 7	182 \pm 8	191 \pm 4	178 \pm 5	195 \pm 5	192 \pm 3	197 \pm 3	182 \pm 7
	100	187 \pm 4	196 \pm 7	204 \pm 6	188 \pm 7	193 \pm 4	189 \pm 6	211 \pm 8	194 \pm 5
	150	167 \pm 7	170 \pm 3	172 \pm 4	161 \pm 2	170 \pm 2	177 \pm 5	175 \pm 7	157 \pm 2
temporal/occipital cortex	30	110 \pm 4	118 \pm 5	114 \pm 8	111 \pm 8	120 \pm 2	114 \pm 4	115 \pm 6	112 \pm 6
	60	102 \pm 3	108 \pm 4	103 \pm 3	101 \pm 5	103 \pm 3	108 \pm 3	109 \pm 5	100 \pm 2
	100	92 \pm 3	97 \pm 4	96 \pm 3	92 \pm 2	92 \pm 2	98 \pm 4	105 \pm 5	91 \pm 2
	150	87 \pm 4	90 \pm 4	90 \pm 3	84 \pm 1	89 \pm 1	90 \pm 3	90 \pm 3	86 \pm 1
midbrain	30	29 \pm 1	30 \pm 1	30 \pm 1	29 \pm 1	30 \pm 1	30 \pm 1	30 \pm 1	28 \pm 1
	60	26 \pm 1	26 \pm 1	25 \pm 1	23 \pm 1	26 \pm 1	26 \pm 1	25 \pm 1	23 \pm 1
	100	22 \pm 1	23 \pm 1	22 \pm 1	22 \pm 1	23 \pm 1	22 \pm 1	22 \pm 1	23 \pm 1
	150	22 \pm 1	22 \pm 1	22 \pm 1	21 \pm 1	23 \pm 1	22 \pm 1	23 \pm 1	21 \pm 1
brainstem	30	28 \pm 1	29 \pm 1	28 \pm 1	28 \pm 1	30 \pm 1	29 \pm 1	30 \pm 1	28 \pm 1
	60	25 \pm 1	25 \pm 1	25 \pm 1	24 \pm 1	25 \pm 1	26 \pm 1	23 \pm 1	23 \pm 1
	100	21 \pm 1	21 \pm 1	22 \pm 1	21 \pm 1	21 \pm 1	22 \pm 1	20 \pm 1	20 \pm 1
	150	17 \pm 1	18 \pm 1	17 \pm 1	18 \pm 1	18 \pm 1	16 \pm 1	18 \pm 1	18 \pm 1

Data represent mean \pm SE obtained from six animals in each treatment group for each age and sex. Results of multivariate ANOVA are provided in the main text. Note that the assays for each region and age were run in separate experiments, so absolute values cannot be compared strictly across ages or between regions. Accordingly, statistical comparisons in the main text were conducted on log-transformed data, which evaluates the treatment differences as a proportion to control values, rather than as an arithmetic difference. Representing the data as proportional differences (percent control) enables a full comparison of treatment effects and treatment interactions with all the other variables, even though absolute values for the controls cannot be compared across regions and ages.